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Organic Growing Media from Tea Waste for Orchids in Kalimantan

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This study investigates the potential of using organic media derived from tea waste and young coconut water for the cultivation of Epidendrum orchids, a rare species native to Kalimantan, Indonesia. The research aims to replace conventional instant tissue culture media (ICM), which contains hazardous chemicals, with environmentally friendly alternatives. The organic media (OM) formulations were prepared by fermenting tea waste to extract nutrients and combining them with coconut water, which is rich in growth hormones. The effectiveness of three different organic media compositions (OM-1 [50ml Compost Extract], OM-2 [100ml Compost Extract], OM-3 [150ml Compost Extract]) was evaluated based on their nutrient content and impact on the growth of orchid explants. Results indicated that the organic media provided sufficient nitrogen, phosphorus, and potassium levels, leading to a 100% survival rate of the explants. Notably, OM-1 demonstrated the highest stem growth (0.46%) and shoot proliferation (2.3) compared to other treatments. This research highlights the feasibility of utilizing organic waste for sustainable orchid cultivation, reducing hazardous waste production, and promoting environmental conservation.
Keywords: Environment sustainability, hazardous chemicals, plant tissue culture, organic matter, sustainable cultivation.

INTRODUCTION

PT. Badak NGL is a liquefied natural gas (LNG) production firm situated in Bontang, East Kalimantan, Indonesia. Despite operating in the fossil fuel sector, PT. Badak NGL actively participates in environmental preservation efforts. The company has a nursery department tasked with promoting greenery and environmental conservation. This nursery has a tissue culture laboratory that propagate many types of indigenous Kalimantan plant seeds, including orchid seeds.

Nonetheless, the tissue culture applies inorganic culture medium (ICM), which relies on hazardous chemicals. The most utilized ICM by nurseries is MS (Murashige & Skoog) media. This substance contains hazardous compounds such as ammonium nitrate, calcium chloride, edetate disodium dihydrate, zinc sulphate heptahydrate, and boric acid. The nursery produces approximately 120 liters of hazardous chemical waste annually.

The preparation of ICM requires extensive glassware apparatus due to the involvement of chemicals. The longevity of the glassware depends on its age and quality. The frequency of glassware breakage at PT. Badak NGL's nursery department is approximately 1 kg per year. The glassware fragments can injure individuals if discarded without protective packaging or proper handling procedures.

To address these issues, the nursery initiated research to substitute ICM with organic media (OM). The OM developed in this research uses compost and young coconut water as raw materials. The OM derived from tea waste and coconut water will support comparable or superior growth of Epidendrum orchid explants compared to ICM. Compost is an attractive option due to its high organic matter content and abundant nutrients (Farrell & Jones, 2010). In addition, compost can help contributes diseases (Hoitink et al., 1997; Noble & Coventry, 2005; Ros et al., 2005; Van der Gaag et al., 2007)

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and increases water holding capacity, and enhances soil fertility, which collectively contribute to better plant growth and yield (Adugna, G., 2016).

Several types of composted materials have been explored as raw materials for organic media. For instance, rice hulls have been employed in the south eastern United States (Evans, M.R., 2011; Evans & Gachukia, 2007; Gomez & Robbins, 2011), while almond shell waste has been utilized in Spain (Valverde et al., 2013). Other plant-based composts, such as bracken (Pitman & Webber, 2013), corn waste (Vaughn et al., 2011), cotton waste (Zaller, 2007), sugarcane waste (Webber III et al., 2015), hazelnut husk (Dede et al., 2011), and gorse (Iglesias-Díaz et al., 2009) have also been studied for formulation as organic growing media.

The compost utilized in this research was derived from tea waste. Tea is an agricultural commodity in Indonesia, which contribute to economy, with the country ranking among the world's top tea producers and generating thousands of tons annually. However, the production also generates a large amount of tea waste – remnants of tea leaves generated during the tea brewing process – that is frequently discarded. The increasing production of tea generates a large amount of tea waste, which, if not managed properly, can cause serious environmental problems (Debnath et al, 2021). However, tea waste also has great potential for beneficial use due to its richness in organic compounds (Zaini & Syafi, 2021).

Several studies show that compost media from tea waste has been successfully used as a planting medium. For example, (Werghemmi et al., 2022) showed that adding tea waste increased the growth induction index value of *Pleurotus ostreatus* (Oyster Mushroom) plants by 17.4%. Similarly, (Gammoudi et al., 2020) reported that the use of tea waste compost improved the final germination percentage and root length of *Capsicum annuum* L. plants. The investigation by (Zaini & Syafi, 2021) also showed that water spinach growth was significantly improved by the combination of 50% conventional soil and 50% tea compost compared to conventional soil.

In this research, organic media based on leaf waste will be tested for cultivating *Epidendrum* orchid seeds. This orchid is a rare native orchid from Kalimantan and is listed as protected flora under the Regulation of the Ministry of Environment and Forestry of the Republic of Indonesia Number P.106/MENLHK/SETJEN/KUM.1/12/2018. The effect of various organic media compositions on the growth of *Epidendrum* orchids will be evaluated in this research including explant height and number of shoots.

MATERIALS AND METHODS

***Materials*:** The compost in this research was obtained from tea waste at the PT Badak NGL Nursery. The compost is dissolved and fermented first prior to use as an OM. The fermentation is carried out for 4 weeks with addition of organic materials such as fruit and vegetable leftovers. The purpose of this dissolution and fermentation is to release Nitrogen, Phosphate and Potassium (NPK) compounds into the water to create compost extract. Meanwhile, young coconut water in this study was obtained from the PT Badak NGL Community Area. PT Badak NGL Community Area refers to an area or program designed to increase interaction and relationships between the company and the surrounding community. This area often focuses on various activities aimed at supporting the social and economic development of local communities. Young coconut water can contain up to 17% potassium (Hamilton et al., 2024). In addition to its high mineral content, coconut water has a sugar content of 1.7% to 2.6% and a protein content of 0.07% to 0.55%. Other minerals include sodium (Na), calcium (Ca), magnesium (Mg), ferro (Fe), cuprum (Cu), phosphorus (P) and sulfur (S).

***Preparation of organic media*:** As seen in Figure 1, the first step in creating OM was to weigh 250 grams of compost and mix with 15 grams of sugar. The mixed material was evenly put into a compost filter. The compost filter must be placed in a bucket with 1.25 liters of distilled water for a day. After one day, the compost extract was heated at 250°C. Mix 250 ml of distilled water with different volumes of compost extract: 50 ml, 100 ml, 150 ml. Ten grams of sugar and 62.5 ml of young coconut water were added to the homogenous compost extract solution. The degree of acidity stays within the range of 5.5–5.8. NaOH 1N is added if the pH of the solution is less than 5.5; if it is more than 5.8 then HCl 1N is added. Three hundred seventy-five grams of technical agar and distilled water were added to 500 ml when the pH falls within the suitable range. The solution was then boiled at 250°C. The solution was then covered with plastic and filled into 25ml glass bottles. The solution was then sterilized for 20 minutes at 121°C and under pressure of 15 psi in an autoclave. Beside sterilization using

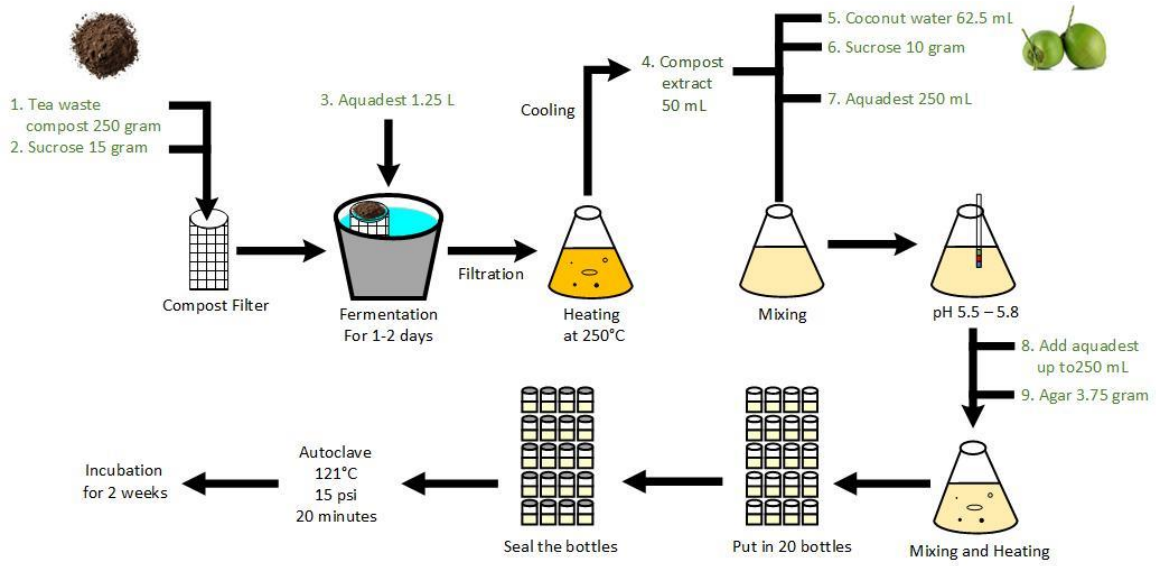

Figure 1. Flowchart for the preparation of organic media.

chemicals and temperature, precaution steps and aseptic handling is very important to maintain sterile condition (Matzain et al., 2023). Before subculture, the final medium is kept in the incubation area for 1-2 weeks.

***Cultivating orchids in organic media*:** The subsequent procedure involves the cultivation of orchids in OM. The culturing procedure is conducted within a Laminar Air Flow Cabinet (LAFC). The initial stage in this procedure is to prepare the LAFC for utilization. The procedure commences with the sterilization of all instruments, including dissection tools, petri dishes, and glass bottles, utilizing an autoclave at 121°C and 15 psi for 30 minutes. Additional materials, including rubber, plastic, and organic media, are sterilized by applying a spray of 70% alcohol. Following the sterilization of the instruments, they are placed into the Laminar Air Flow Cabinet (LAFC) together with 95% Bunsen alcohol. Subsequently, activate the LAFC UV light and the UV lamp in the workspace for one hour. After one hour, both UV lights were deactivated and the bunsen burner was activated for 30 minutes. As shown in figure 2, before placed into the sealed bottles, explants were aseptically retrieved from culture bottles using forceps that had been pre-cooled to minimize tissue damage. The explants were transferred into sterile Petri dishes, where clumped tissues were carefully separated into individual explants using a scalpel (George et al., 2008). Each isolated explant was then transferred into a sterile bottle containing organic culture media. The bottles were sealed with sterile plastic lids to prevent contamination. Finally, all prepared culture bottles were placed inside a LAFC. Variations in the composition of the OM were carried out as shown in Table 1. The effect on explant height, number of shoots and physical condition was observed every week.

Effect of media composition to explant growth

Several parameters were measured to assess medium quality and explant growth such as nutrient contents, stem and shoot growth, life percentage and health of explants. Measurement was conducted 20 days after propagation and every 1 week after until 19 weeks.

Figure 2. Cultivating orchids in organic media.

RESULT

The results indicate that OM-1, OM-2, and OM-3 contain nitrogen, phosphorus, and potassium levels comparable to

Table 2. Organic media composition.

Parameter	% adb			
	ICM	OM-1	OM-2	OM-3
Total phosphorus as P_2O_5	0.012	0,028	0,017	0,021
Potassium as K_2O	0.01	0,008	0,0088	0,0097
Total Nitrogen	0.18	0,19	0,13	0,14

those of chemical media (ICM). As presented in table 2, the largest nitrogen and phosphorus content is found in OM-1 at 0.19% and 0.28% %.

***Explant Life Percentage*:** The investigation was conducted with four distinct treatments. Each treatment was conducted 20 times, resulting in 80 explants per orchid plant. Table 3 indicates that Epidendrum orchid explants exhibit a survival rate of 95 to 100%. Epidendrum orchid explants can survive in OM due to the presence of sufficient nutrients. The outcomes of live explants exhibit favourable germination rates, vibrant foliage, and robust root systems (figure 3). According to the research findings, explants that exhibited contamination were exclusively identified in ICM. The contamination of Epidendrum explants in ICM is believed to result from inadequate sterility, allowing contaminants to infiltrate the culture bottle. The origin of pollutants affecting explants is a white fungus situated surrounding the explant, which proliferates into the growth media as the planting duration extends.

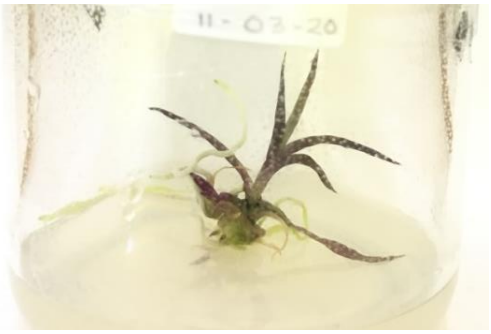

Figure 3. Healthy Epidendrum Explants

The browning phenomenon transpired in Epidendrum explants within OM-1 and OM-2. The discoloration of the explants was attributed to the use of tweezers that remained heated post-sterilization over a Bunsen flame, along with an extended oxidation process during operations in the LAFC. Additionally, the browning of explants may result from improper separation or cutting of the explants within the culture bottles. Browning may result from the action of copper-containing oxidase enzymes, such as polyphenol oxidase and tyrosinase, under oxidative circumstances following tissue injury (Amente & Chimdessa, 2021). Figure 4 illustrates the browning of Epidendrum explants.

Figure 4. Epidendrum explants exhibit browning

Shoot and Stem Growth

This study monitored the weekly percentage of stem development. This value was determined by using equation (1). Table 4 indicates that Epidendrum explants cultivated in OM-1 had a superior stem growth percentage of 0.46% relative to other organics media. Comparable outcomes are also seen from the increased number of shoots, specifically 2.30. This is due to OM-1 possessing the largest concentrations of Nitrogen and Phosphorus relative to other organics media.

$$\%\ stem\ \text{growth} = \frac{\text{Final stem height} - \text{Initial steam height}}{\text{Days plant alive}} \text{ x } 100\% \quad (1)$$

Table 4. Growth of shoots and stems of Epidendrum explants in the 19th week post-planting

Treatment	Stem growth (%)	Number of shoots
OM-1	0.46	2.30
OM-2	0.37	1.90
OM-3	0.40	2.00
ICM	0.52	4.20

Utilizing compost tea extract as a growth medium in tissue culture activities supplies both macro and micronutrients, along with nourishment for explants. The inclusion of coconut

water in the media significantly enhances the growth of Epidendrum explants. Coconut water contains hormones such as auxins, which facilitate stem and shoot growth in explants, and cytokinin, which promote cell division by enhancing protein synthesis to fulfil the requirements of the explant. (Ansar & Paiman, 2022) demonstrated that the application of 100 ml of coconut water can significantly enhance the growth of shoots in vitro. The growth of Epidendrum explant shoots is illustrated in Table 5.

Tabel 5. Development of Epidendrum explant shoots at 1-week post-planting (WPP) and 19 weeks post-planting (WPP)

Treatment	1 WPP	19 WPP
OM-1		
OM-2		
OM-3		
ICM		

This research indicates that chemical media yields the greatest stem growth and the largest shoot proliferation. Nevertheless, the disparity in the percentage of stem development and the number of shoots with MO is not substantial. Furthermore, the implementation of MO in the Nursery sub-unit can reduce production costs by Rp. 288,000, with a capacity of 300 planting media each month. From an environmental standpoint, the utilization of organic medium can decrease hazard waste production by 10 litres per month.

DISCUSSION

In this study, in vitro growth of Epidendrum explant has been investigated in 4 different media. Epidendrum explants showed not only high survivability but also healthy growth in organic media processed from tea waste. This finding showed that agricultural by-products such as tea waste could be used for alternative media rather than conventional ones. This successful usage of tea waste as growth media proved its viability as environmentally and cost-efficient option for large scale for plant propagation.

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***Chemical properties of Organic Media*:** Chemical properties are important in providing nutrition for explant growth. In this study OM-1 was the closest to ICM regarding nutrient contents. According to (Crasta & Bopaiah, 2018) NPK was needed for boosting explant seedling, resulting in good rooting. Media based on organic waste has positive impact on explant growth, this is regarding better stimulation in organogenic and embryogenic ability of explant (Wiszniewska et al., 2023). Compost also contains organic acids such as humic and fulvic acid that promote plant growth (Kashyap et al., 2019).

Tea waste contains high amount of lignin and hemicellulose which with proper composting process can be decomposed to nitrogen, phosphor, and potassium-plant primary macro nutrients essentials for growth. Composted tea waste is rich in various nutrients such as N, P, K, Ca, Mg, Na, Fe, and Zn useful for explant growth, with 19 – 21 C/N ratio, theses nutrients can be absorbed quickly then promoting plant growth (Ross & Joothi, 2015). Gupta et al. (2020) added that tea waste compost from green tea dust also increased water holding capacity in soil, making water available for longer period than without compost. With C/N ratio of 11-30 along with high organic carbon and high nitrogen content, it is clear that tea compost waste is ideal for plant growth medium.

Coconut water role as additive substance plays supportive role as nutrients and hormones source. According to study by Mardhikasari and Yunus (2020) coconut water promoted explant growth by providing nutrients and enzymes for growth. Coconut water contains high nutrients and high cytokine level, useful for cell division and organ formation (Khalifah & Rasdianah, 2019). In addition, coconut water also contains macro and micronutrient such as N, P, K, Fe, Mn, B, Zn for regulating metabolisms, flowering, energy transport (Norrizah et al., 2023). Yong et al. (2009), noted that coconut water was widely known as natural additive for growth medium because it contained phytohormones like cytokinins, auxins, gibberellins and also amino acid that played role in cell division and development and organogenesis process in in vitro medium. Moreover, George et al. (2008) reported that coconut water exhibited antioxidants that helped combat oxidative stress during in vitro propagation, thus increasing survival of explants.

***Effect of Media on explant life percentage*:** Life percentage in OM media were 100%, this indicated sufficient nutrient and growth factors in these media. Organic media is rich in carbon which is very important for cell growth (Setiaji et al., 2020). Study by (Ostojić et al., 2022) showed that organic media had shorter time and higher percentage of germination for Orchid in vitro cultivation. In ICM the 95% life percentage could be attributed to unsterilized procedure during propagation. This can be prevented by soaking explant in Mercuric chloride (HgCl2) and Fungicide in short duration of time (Pandey & Prakash, 2022). In addition, study by Loffredo et al. (2009), humic acid in compost acted as natural pesticide to combat plant pathogens in in vitro experiment.

Life percentage in explant was also attributed to nutrient availability. Content of substance consisting of macro nutrients, micro nutrients, and growth regulator are very important for in vitro propagation (Harahap, 2014). Study by Yuliani et al. (2021) stated that explant needed more than 1 growth hormone to reach optimum growth, for example auxins and cytokines were needed for optimum callus induction and biomass.

Though organic media have 100% life percentage but browning case is still high (15-25%). Browning in explant is natural causes by polyphenol oxidase (Chuanjun et al., 2015) in response to tissue damage when slicing explant (Permadi et al., 2024). A review study by Permadi et al. (2024) suggested that browning in explants can be minimized by incorporating anti-browning agents into medium such as Active charcoal, Polyvinylpyrrolidone, Ascorbic acid, and Sodium nitroprusside. Explant deaths were also attributed to contamination by microorganisms. This was supported by Septianingsih et al. (2024) that contamination can happen by high microorganism population in humid air or unsterilized tools and medium.

***Shoot and stem growth of Epidendrum explants*:** It is clear from the study that tea waste could be the alternative for explant media. It had result near to ICM in terms of stem growth. This was supported by study of Tarashkar et al. (2023) that tea waste compost treatment provided better taproot and leaf growth in radish than normal soil medium. Study by Gonbad et al. (2013) stated that tea waste compost gave best result in survival percentage and leaves number, this attributed to optimum pH, increased water holding capacity, and stimulated microbial activity. Addition of coconut water had better impact on explant growth, this was proven by Ostojić et al. (2022) that medium with coconut water addition had better height and width than normal medium with no addition.

Beside stem growth, shoot number is also affected by nutrition and growth hormones. Organic media contains organic acids, cytokinin, and other growth hormones that promote plant growth. This was supported by Lee et al. (2022) media with organic additives provided better shoot height and number in Ficus carica in vitro regeneration. Coconut water is also rich in phytohormones like auxin and cytokinin. Harahap et al. (2023) stated that shoot formation was relied on high cytokinin and low auxin, with proper concentration of these two hormones, explant growth could be optimized. Jainol and Gansau (2017) argued that coconut water addition increased Protocorm Like Bodies (PLB) and shoot multiplication caused by growth hormones, vitamins, amino acids, and minerals.

ICM highest shoot number caused by explant orientation, it was stated by Prando et al. (2014) that explant in horizontal orientation had more shoot number than explant in vertical orientation. Aside from that, size of explant cutting affected how explant growth in vitro. This was stated by Arli et al. (2023) that thinner explant cutting resulted better growth because thinner cut facilitated better contact and nutrient diffusion with medium.

***Conclusion*:** In conclusion, Organic media showed best result with 100% germination ratio. In terms of stem growth and shoot number, OM-1 was the greatest among other organic media treatment. This study indicated that utilization of

organic waste showed great potential in ecological and economically efficient in vitro cultivation.

***CRediT author statement*:** Yosvaldo Gerry Setiono Putra created the methodology and conducted experiments, Maulana Ardy Vilyant validated data and authored the initial draft, Mufti Cahyanto contributed to the investigation and conceptualization, Edi Lukito oversaw the project and validated the data, Novesa Nurgirisia performed a review, Bardhian Cahyo Aji Gumilang reviewed and drafted the manuscripted, and Firman Asto Putro composed the final manuscript

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***Policy referred*:** United Nations 2030 Agenda for Sustainable Development (2015); Convention on Biological Diversity (CBD, 1992); Indonesia's National Biodiversity Strategy and Action Plan (NBSAP)

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